THE INFLUENCE OF SOME OF THE [illegible] ORGANIC [illegible] Y MATERIALS ON THE INT[illegible] AND PHOTOSYNTHESIS OF WI[illegible] FOLIAGE

by

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INTRODUCTION

Twentieth century commercial horticulture, in a competitive trade, has learned to think and to act seriously about insect pests and diseases. The home orchardist with his sprinkling of fruit trees likewise finds it no longer possible to enjoy the harvest of premium fruit without a watchful eye and timely prevention of pest attacks. Childers (1949) reports that 20 to 40 percent of the total cost of tree fruit production is devoted to insect and disease control.

Scientific horticulture through the years has employed the strategy of spray material application for pest control. The 1949 fruit grower will report as a common practice 15 or more applications of spray materials for apple fruit and tree protection. To achieve his goal the horticulturist employs the methods and materials of the chemist, the plant physiologist, and the physicist in his tactical maneuvers.

The years 1940 to date have produced speedier, less costly and more efficient equipment for applying chemical spray materials. These same years have produced from the laboratories of the organic chemist a whole new galaxy of spray materials generally referred to as the organics. Manufacture of these substances has produced a maze of trade names claiming outstanding results for use as spray materials. Along with the recognition of their beneficial effects, enough evidence has accumulated to question the effects of the new materials on the plant tissue to which they are applied. The effect upon pests has been substantiated with research of both

public and private laboratories. The professional horticulturist now demands to know whether the plants also suffer along with the pest.

The query is not easily answered. Plant health is not readily measureable. One method of research, however, measures the effects upon those vital life processes which are to some extent measureable. Pickett and Birkeland (1941) have suggested another measuring stick, the depth of the palisade layer of the leaf anatomy. Accordingly, the intent of this problem has been a measurement of the effects of four of the newer organic spray materials upon healthy apple foliage. Specifically, Fermate, Parethion, Chlordane, and 2,4-D have been tested for their influences upon the process of photosynthesis and the internal structure of the Winesap apple leaf grown under Kansas conditions, 1948.

LITERATURE REVIEW

Photosynthesis

Photosynthesis, sometimes referred to as carbon assimilation, is as vital to the continuance of life as are the various human life processes. Its end products energize commerce and life itself. Without it or its source of radiant energy life on earth would cease.

Concept. Twenty years ago science regarded photosynthesis as a presumably complex one-step process. 1949 records van Neil (1949) writing in terms of the still complex "comperative biochemistry of photosynthesis" and asserting the importance of both a photochemical ("light"), and a non-photochemical ("dark") portion of the process. The early formula representing the measurements of de Saussure in 1804 is the classic one of the general botany classroom:

$$CO_2 + H_2O \xrightarrow{Light} (CH_2O) + O_2$$

van Neil in his summarization of the researches leading to the present concept concludes that the present-day formula should be:

$$CO_2 + 4H_2O \xrightarrow{Light} (CH_2O) + 3H_2O + O_2$$

In both formulas the (CH_2O) indicates an indefinite organic product.

Photosynthesis as defined by biochemist van Neil is "a process in which carbon dioxide is photochemically reduced to organic matter with the simultaneous oxidation of a reducing agent." Physiologist Miller (1938) defined the process as "the manufacture of some simple carbohydrate (probably sugar) from

carbon dioxide and water by the chloroplasts in the presence of light." In this process oxygen always appears as a waste or end product.

The leaf of the Winesap apple tree, Malus pumila, is the site of the process considered here. Chloroplasts containing energy-absorbing chlorophyll stream around within the cytoplasm of cells largely within the palisade and sponge tissues. The chloroplasts are small green bodies, quite uniform in size and shape, usually disks or flat ellipsoids three to ten millimicrons across, and varying in number from a few to a hundred or more, (Rabinowitch, 1945). Since the time of Engelmann and Reinicke it has been considered that the reaction sequence of photosynthesis begins and ends in the chloroplasts, although Rabinowitch emphasizes the necessary cooperation with the protoplasm.

Factors Affecting the Process. The complexity of the problem at hand mounts as additions to knowledge increases. Blackman (1905) introduced the concept of "limiting factors." "When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the slowest factor." He also recognized five obvious factors controlling chloroplasts engaged in photosynthesis: 1) the amount of carbon dioxide and 2) water available, 3) the intensity of the available radiant energy, 4) the amount of chlorophyll present, and 5) the temperature within the chloroplast. Miller (1938) presented them as internal and external factors and added protoplasm as a co-internal factor with the chloroplasts. Rabinowitch

(1945) in volume one of a proposed two-volume work in photosynthesis adds to the list of factors. Pertinent observations or hypotheses for each of the factors follow.

Light. Under Kansas conditions Pickett (1934) found that the period from 6 a.m. to 2:30 p.m. was the period of greatest gain in dry matter. In 1933 he had reported 8:30 a.m. to 5:30 p.m. as a satisfactory period for sampling. Hyre (1939) found that the second 6½ hour period of the day had a lesser rate of apparent photosynthesis than the first period. Yields reported by Heinicke (1932) were higher in the morning hours with high values also a few hours before sunset. Five-hour periods on bright days yielded three to five times as much photosynthate as on warm clouded days. Heinicke and Childers (1937) found that the five hours of greatest light intensity produced about 47 percent of the daily photosynthate. Evidence suggested that on clear nights during the period of full moon, apparent respiration was less than on totally dark nights.

Heinicke and Childers (1936) have found that there may be enough light after 7:30 p.m. and before 4:30 a.m. to offset respiration. During June and July they recorded many nights in which photosynthesis actually exceeded respiration. They recognized, also, a trend of decreasing photosynthesis as the season advanced, corresponding largely to the gradual decrease in daily light intensity. The daily transpiration curve indicated a close relationship to the light curve. Both were higher during the early part of the season than during the latter part. Highest

rates of apperent photosynthesis were essociated with high light intensities, moderate mean temperatures, and relatively low transpiration-assimilation ratios.

Heinicke and Childers (1936) noted the following light measurements during July 26, 1936 at Ithioe, New York:

9200	footcandles	- light target toward the sun
8100	"	- horizontal
350	"	- shaded leaves one foot in from south side
320	"	- shaded leaves, north side
80	"	- interior leaves eight feet above ground
40	"	- four feet above ground

Christopher (1933) measured light intensities on different sides of a tree and found that leaves exposed to the morning sun are the best equipped to use sunlight efficiently considering water, carbon dioxide, and temperature. Greater light intensities were generally found on the east side. Lubimenko, as reported by Miller (1938) has noted that shade plants with lower intensity of illumination can accomplish the same amount of photosynthesis with a lower illumination than can sun plants. Shade plants contained the higher chlorophyll content. However, they were less efficient in high light intensities than were the sun plants.

Franck and French (1941) assumed that light causes an inhibition of photosynthesis by the photooxidation of one of the photosynthetic enzymes.

Temperature. Heinicke and Childers (1936) reported that highest rates of assimilation rarely occurred at higher temperatures. Actually, high mean temperatures greatly reduced photosynthesis. In 1937 they concluded that the rate at a given light intensity should be highest when the temperature is lowest.

Conversely, a low photosynthetic rate results from a high temperature at a given light intensity. The data also revealed that the daily rate did not fluctuate but proceeded as actively in the afternoon as in the morning. The writers in 1936 stated that the most favorable condition for accumulation of carbohydrates is during the close of the growing season when the weather is cooler and less favorable for high respiration in spite of the less favorable conditions for photosynthesis.

Carbon Dioxide. Heinicke and Childers (1937) concluded that no marked relationship exists between different rates of apparent photosynthesis and the carbon dioxide content of the air at any given light level. High carbon dioxide content occurs at high light intensity perhaps due to a higher temperature favorable for decomposition processes. The carbon dioxide content was usually highest on the warm cloudy nights and lowest on the cool, clear, and windy nights. In 1932 Heinicke observed assimilation rates per unit leaf area of plants the same age and variety varied as much as 500 percent under similar external conditions. He attributed differences to internal conditions such as water supply, nitrogen, chlorophyll, vigor of plant, etc. Christopher (1933) has recommended that an air flow of 2 to 2.5 liters per cm sq per hour be used in experiments to approximate natural conditions. In 1938 he noted that normal leaves on thin wood and on thick wood assimilated carbon dioxide at similar rates.

Dehydration. As a rule dehydration decreases the rate of

photosynthesis. Rabinowitch (1945) reported the following work. In 1935 Alexeev found that photosynthesis of apple leaves is at a maximum at twenty-eight percent water deficiency. Brilliant and Chrelashvili in 1941 discovered that only high light intensities caused a stimulating effect of a moderate dehydration. Strong dehydration, however, inhibits the process in both weak and intense light. Daetur found the maximum rate of photosynthesis within any species was in proportion to the water content of the leaf. Because the stomata close in dry air, the real cause of the decreased rate probably lies in the reduced rate of carbon dioxide availability to the internal leaf surface.

Age of the Tissue. Heinicke and Childers (1937) experimented with a living tree having 10,000 leaves during 188 days of the growing season, 1935. They found that the maximum daily rate of photosynthesis was obtained as early as June 25. Prior to that date the rate had increased slowly from a dormant-season deficit. For some time the tree was unable to assimilate as much as it was respiring, but with rapidly increasing leaf area, especially after bloom, the rate increased rapidly. The trend of assimilation is largely determined by the extent of available leaf surface. After July 1 the effective leaf area probably is not increased.

Howland (1946) reported that co-worker Eliot found old leaves equally efficient as newly-matured leaves in food syntheeis of roses. Singh and Lal (1935) have found that for mature leaves under optimum conditions the photosynthetic value is low while

plants are young, become maximum when plants are at maximum growth and maturity, and slows to zero near the end of the crop year.

Carbohydrates. Warburg in 1919 has concluded that accumulation of carbohydrates is somewhat responsible for decline in photosynthesis after prolonged illumination. Rabinowitch (1945) explained the situation as a "blocking of enzymes or active surfaces or by the diversion of light energy to photo-oxidative processes." This indicates either an inhibition of photosynthesis or an acceleration of reverse processes. Spoehr (1926) considered that the presence of carbohydrate accelerates respiration which in turn increases photosynthesis. Chandler (1934) believed that sufficient evidence exists to indicate that accumulated products reduce the rate of photosynthesis. Heinicke (1932) showed the reduced photosynthesis on a ringed branch and the accumulation of products above the ring. Rabinowitch ascribed to carbohydrate effects the regulating factors, among others, species, light intensity, temperature, and form of accumulation.

Inorganic Elements and Ions. Inorganic ions show both direct and indirect influences on photosynthesis. Rabinowitch (1945) listed them as direct catalytic effects or indirect deficiency effects. The potassium ion is both an indirect deficiency as in chlorosis and also is a direct stimulus to the rate of photosynthesis. Magnesium, iron, manganese, nitrates, and phosphates comprise the list of other known indirect ionic effects.

Ionic inhibitory substances include hydrogen and hydroxyl ions, alkali ions, heavy metal ions, and anions. Greenfield (1941, 1942) has noticed inhibiting effects of iodides and borates in the presence of strong light on Chlorella.

Oxygen. Rabinowitch (1945) reported that the complete absence of oxygen often brings photosynthesis to a standstill. An excess of oxygen consistently reduced the rate of the process. Warburg (1920) and McAlister and Myres (1940) found a decrease of thirty percent in the maximum rate of photosynthesis of Chlorella when in strong light and abundant carbon dioxide within an oxygen concentration from 0.5 to 20 percent.

Catalyst Poisons and Narcotics. Rabinowitch (1945) has presented evidence that photosynthesis is strongly affected by many so-called inhibitors or stimulants, substances which change the rate without participating directly in the reaction. Certain poisons inhibit specific steps in the photosynthetic process. A skillful use of such inhibitors promises to become an important tool in the disentanglement of this complex process. Such poisons include cyanide, hydroxylamine, hydrogen sulfide, carbon monoxide, sulfur dioxide, nitrous oxide, hydrogen peroxide, the iodoacetyl radicle, and several narcotics.

Miscellaneous Chemical Stimulants. Rabinowitch (1945) stated that "it appears as though almost any inhibitor becomes a stimulant if used in sufficiently low concentration." He claimed that although numerous poisons had been found for inhibition of

specific phases, there had been no agent found for an acceleration of the entire photosynthetic process. Rather, such stimulants as have been found are considered "protoplasmic stimulators." He reviewed the work of Kholodny and Gorbovsky, 1939 and 1941, reporting that the rate of photosynthesis of Hydrangea and hemp was temporarily doubled by the common growth hormone, B - indoleacetic acid. Other observations on the effects of chemical spray materials appear below.

The Russian Kostychev in 1921, and later, Lubimenko and Shcheglova in 1933 reported the effect of mechanical injury on photosynthesis. Rabinowitch stated:

> ... by punching holes of known area and comparing photosynthesis per unit surface of wounded and attached leaves. They found a marked stimulation which, however, became apparent only two or three days after the injury. The authors attributed this 'induction period' to the water loss caused by wounding. After the wounds healed stimulation gained over inhibition, and remained noticeable for several weeks. If the perimeter of the wounds was too large, the stimulating effect was pronounced even in the first two or three days; but after this the photosynthesis sank rapidly below the normal level. The wave of photosynthesis after injury ran parallel with a wave of respiration.

Physical Stimulants and Inhibitors. Ultra violet light, electric fields and currents, and radioactive rays still further complicate the process and without doubt will receive further investigation in the future.

Respiration. Heinicke and Childers (1936) have indicated that respiration increases as temperature increases. They suggested that a high respiration during the night may cause an

inhibition on photosynt esis the next day. Their reference to it was a "hang-over" effect. The average rate of night respiration reported consisted of considerably less than ten percent of the average hourly rate of apparent photosynthesis during the day. Their results indicate a doubled respiration during the daytime. Denny (1932) in comparing three methods of measurement, found that loss in dry weight from Lilac was not measureable. Pickett (1934) reported a close correlation between temperature and night respiration, with greatest losses during the warm nights.

Methods of Measurement. There are three general methods discussed by Miller (1938) for measuring the rate or total amount of photosynthesis: 1) the rate of oxygen liberated in the process, 2) the amount of carbon dioxide absorbed, and 3) the amount of dry matter produced. In each method respiration biases the measurement because the end products of photosynthesis are the raw materials for the respiration process which is proceeding at the same time. Pickett (1935) compared the punch method of Sachs, the more modern carbon dioxide absorption methods, and the saccharification method and suggested that there is no one certain measure of photosynthetic rates. He suggested that the method employed be selected for the conditions under which one works.

Workers at the New York State Agricultural Experiment stetion have preferred the carbon dioxide absorption method such as used by Heinicke and Childers (1937). The principle of the method was suggested in 1885 by Kreusler who noted that green leaves have the capacity for carbon dioxide removal from the air. Present-day

methods are varied but the principle remains the same. The leaf surface enclosed within a chamber receives a continuous quantity of fresh air. As air emerges from the chamber it is analyzed for its carbon dioxide content. A decrease in the carbon dioxide content indicates apparent photosynthesis. An increase indicates apparent respiration.

Christopher (1933) has suggested that for comparable reporting, "it is equally as important to guage air flow according to leaf area as it is to report assimilation in terms of mg. per 100 cm^2." He noted that Wilson (1933) had used 4.8 litres of air per hour on six clover plants, whereas Heinicke (1932) had supplied 50 to 100 litres per hour to one apple leaf. He suggested that the rate be standardized at 2.0 to 2.5 litres per sq cm per hour.

Hickett (1937) determined the increase in total dry matter of small trees between the time of planting and the digging five months later. Each tree was weighed before planting. Three trees were killed by heat and oven-dried to determine the percentage of moisture representative of all the trees planted. He used the gain in dry matter as an index to the rate of photosynthesis.

The dry-weight leaf-punch method requires that sample leaves be punched prior to the photosynthetically-active portion of the day and again just following the peak period. The difference between morning and afternoon dry weights of equal leaf area is determined from the same sets of leaves. This represents the net amount of photosynthate remaining in the tissues during the period. Translocation of photosynthate and respiration of other photosynthate have proceeded concurrently with photosynthesis. The dry

weight actually measured, also called "apparent photosynthesis," therefore is only a relative approximation of the actual rate of assimilation.

Sachs in 1884 for the first time measured the photosynthetic rate quantitatively. Using the half-leaf method, he removed one-half of an attached leaf along the midrib and allowed the other half to proceed with photosynthesis during a given daylight period. The difference in dry weights of both halves at the end of the experiment indicated the amount of photosynthate produced during the course of the experiment. Denny (1930) summarized the criticism of this method: 1) the half leaves were not symmetrical, 2) there was shrinkage in area measured, 3) wounding the leaf increased the rapidity of translocation from that portion of the leaf that remains, and 4) wounding leads to pathological conditions.

Ganong (1905) reported that the most conclusive way of demonstrating organic increase through photosynthesis was by the Sachs method. However, he noted the inconvenience of the manipulation, and three years later introduced a leaf punch which removes one square centimeter of leaf area. This development presented a standard for experimental comparisons and decreased the intensity of objections to Sachs' original method. Two years later Thouday (1910) concluded that the dry weight method is satisfactory for change in rates greater than two milligrams per square decimeter per hour.

Denny (1930) introduced the "twin-leaf" method of measurement

which takes advantage of the fact that opposite leaves on plants are under more identical conditions of growth than are two half leaves. In 1933 he objected to the dry weight measurement, for his results were not quantitatively and sometimes not qualitatively correct. The presence of dew on the foliage eliminated the use of fresh-weight data. Howland (1946) reported his success with Denny's method on roses. He cited Curtiss' tests acknowledging the validity of the method.

In the use of the dry-weight method, as with the other measurements, the selection of representative leaves is important. Pieniazek and Christopher (1944) used only the sixth and seventh leaves from the top of a tree pruned to a single vigorous stem. Hoffman (1933) worked with average-sized leaves on the east or southeast side of the tree about four to five feet from the ground. This position occurred about the middle of terminal shoots well exposed to light.

Boynton and Compton (1944) selected fifty leaves at random from the middle portions of shoots on the outside of trees. In 1945 they noted that chlorophyll content decreased with increasing age of the leaf, and so they suggested that leaves be selected from the middle portion of shoots. Pickett (1933) sampled leaves from outer shoots on the south side of the tree.

Heinicke and Childers (1937) reported that leaves shed by the middle of October had been formed early in the season and were fully expanded by early June. However, being on the basal parts of shoots, they received less sunlight as new leaves appeared.

Effects of Spray Materials. Horticultural literature abounds with experimental results of chemical spray application. Representative reports follow below.

Insecticides and Fungicides. Clore (1936) reported no effect on photosynthesis by Bordeaux sprays as compared with untreated leaves. Hoffman (1932), however, reported that Bordeaux-sprayed leaves tend to regain their former efficiency after the precipitate has been washed off. He believed that Bordeaux causes a physical effect only and not a chemical disturbance such as lime-sulfur produces.

Bordeaux as a control for apple scab was surpassed by lime-sulfur in 1907 with less tree injury. Lime-sulfur has since continued to be the standard fungicide on apples for over thirty years. The Bordeaux had often severely defoliated trees and caused much russeting of the fruit. At times such injury was more severe than the disease.

Eyre (1939) has concluded that injury from lime-sulfur was due to decreased photosynthesis rather than to increased respiration. His observations were based on tests in which only the lower sides of leaves were sprayed. Hickett and Birkeland (1942) found that liquid lime-sulfur reduced the amount of internally-exposed surfaces more than did lime sulfur. Hoffman (1933) reported, however, that not all experiments with lime-sulfur produced injury.

Mitchell and Childers (1944) received reports from Ohio growers that Kolofog-sprayed leaves were larger than leaves sprayed with other sulfurs. Tests indicated Kolofog as stimulating to

apple leaf growth. Kolofog, a milder spray than lime-sulfur, is fused sulfur absorbed into Bentonite. Agnew and Childers (1939) reaffirmed the results of other workers with the conclusion that sprays containing sulfur in suspension have less effect upon photosynthesis of apple leaves than sprays which contain sulfurs in solution. Rasmussen, Toenjes, and Strong (1948) reported that trees sprayed with less caustic materials such as wettable sulfur, proprietary coppers, and some organic fungicides were as free from disease and insect injury as lime-sulfur-sprayed trees. The lime-sulfur caused reductions in yield from 27 to 53 percent. Hyre (1939) tested the shading effects of spray materials with a transparent bag and found that flotation sulfur did not reduce photosynthesis. Christopher (1935) found the flotation sulfur safer than lime sulfur as an apple scab spray.

Hoffman (1932) suggested that reduction in leaf activity due to lime-sulfur applications varied with the weather but also varied more with the internal leaf condition itself. In 1933 he considered high temperatures as the most potent environmental force associated with injury. Hyre (1939) suggested that an increase in injury from lime-sulfur resulted from changing from overhead applications to heavy driving applications from the ground. He also reported a reduction in assimilation due to shading effect of the spray deposit comprising hydrated lime, arsenate of lead, and a sulfur fungicide.

Using lime sulfur spray on a ten-year old tree, Heinicke (1938) reduced the rate of photosynthesis by forty nine percent immediately after spraying. That rate was maintained for five

days, and later, when back to normal, a second spraying reduced the rate by twenty eight percent. Hoffman (1933) reported that with lime-sulfur the more severe cases of reduced efficiency were usually accompanied by serious marginal burning two or three days after spraying. The reduction in assimilation showed up the first day. In 1932 he had reported as much reduction in the rate two weeks after spraying as on the second day, using lime-sulfur.

Hoffman (1933) has noted that where considerable leaf injury occurred the lime-sulfur spray had been applied during the early afternoon about two or three o-clock. In a companion experiment there was no injury where the application had been unintentionally delayed until late afternoon, about 6:30 to 7:00 p.m. Reductions in the assimilation rate, according to Hyre (1939), ranged from zero at 70 degrees and 10 percent at 85 degrees, to 25 percent at 100 degrees farenheit, using four sulfur treatments.

1938-1939 tests by Rasmussen, Toenjes, and Strong (1948) first indicated the holdover effects of fungicides upon fruit-bud differentiation and the yield the season following application. In their 1944 tests the lime-sulfur trees had a slight bloom as compared with the heavy bloom of less-caustic materials.

Pieniazek and Christopher (1944) found that Fermate is much safer than lime sulfur even at high temperatures. Spraying with Fermate and lime during cool weather produced less injury than did the lime-sulfur. Application of Fermate was at the rate of 1½ pounds to 100 gallons of water by means of a small electric paint sprayer. Braun (1947) reported that Fermate-sprayed grapes increased vine growth and yields as compared with a decrease in

growth and yield when sprayed with Bordeaux.

Yeager and Rasmussen (1948) have reported that Formate-sprayed Northern Spy trees remained uninjured following early autumn frost, whereas trees sprayed with ordinary mild sulfur were badly injured. Dutton and Wells (1923) reported more frost injury to Bordeaux-sprayed trees than to lime-sulfur sprayed foliage.

Hoffman used a summer oil in two tests and observed an immediate and considerable reduction in assimilation in the greenhouse. After a week, however, there was complete recovery. He also noted that light green leaves, both sprayed and unsprayed, were more injured from high temperatures than were the dark green leaves. Schroeder (1935) reported an increased reduction in assimilation following the second oil application. Moon and Harley (1946) found that leaf efficiency was unimpared by DDT residue on apple foliage.

Laurie and Witt (1941) tested greenhouse roses and found that marked reductions in assimilation rates followed the first day of application, using a rotenone and pyrethrum spray. A gradual recovery commenced on the fifth or sixth days. A second application reduced the initial change even greater, but the same recovery took place.

Growth Regulating Sprays. Harvest sprays, as reported by Rasmussen, Toenjes, and Strong (1948), became effective 72 hours after application and were effective for seven to eight days. One spray of 20 parts per million was as effective as two applications

of 10 parts per million. The higher concentrations became effective sooner. These workers reported that early dropping of fruit causes a greater loss to the grower than does the injury from scab, coddling moth, or spray injury. Especially during harvest periods of high temperatures, these sprays are extremely valuable. Harley, Weon, and Regeimbal (1947) have found 2,4-D at 10 ppm highly effective for two consecutive years with long effective periods for both Stayman Winesap and Winesap. Salts and esters were highly effective, with the butyl ester of highest intensity. However, it also advanced fruit maturity. When a heavy rain occurred about one hour following spray application, the effectiveness of 2,4-D was decreased.

Batjer and Thompson (1946) recommended 2,4-D spray on Winesap foliage no greater than 10 ppm, 20 ppm on McIntosh showed no visible injury. On Bartlett pears, however, concentrations above 5 ppm were injurious. Carbowax increased intensity of effects of naphthaleneacetic acid sprays. The writers found that 2,4-D on Winesap was much superior to naphthaleneacetic acid both in intensity and duration of effect. Its effectiveness on Winesap and not on other varieties is peculiar to 2,4-D. Marsh and Taylor (1947) warned of a cumulative effect resulting in tree injury from annual use of 2,4-D. They reported a ten-month lasting effect of this chemical and also a selectivity for Winesap, Stayman, and possibly Northern Spy varieties. Fridham (1947) failed to obtain symptoms of 2,4-D injury from spraying dormant twigs in winter and suggested that absorption through the leaf or other succulent tissue is necessary.

Blossom-thinning sprays have produced so varied results that the methods and techniques of one area are not applicable to another, according to Butjer and Thompson (1948). They have found that the center or "king blossom" is more resistant than side blossoms to the action of dinitro, Naa, and PTD sprays, resulting in a larger percent of fruit arising from those blossoms. Naa sprays produced a flagging of foliage for five to ten days after treatment without dwarfing or epinastic effects. In some cases, reduction in fruit set following full bloom spray occurred only after three to five weeks following bloom. DN-2 sprays have been reported to produce a crinkling and mottling of foliage.

Leaf Structure

MacDaniels and Cowart (1944) have presented the most extensive review of Malus pumila leaf morphology. The following discussion includes much of their experimental conclusions.

External. The visible exterior of the leaf consists of the expanded leaf blade and petiole with vascular elements within the petiole radiating out pinnately into the blade. On the underside of the blade the surface is ribbed with vascular elements and is slightly pubescent. The topside is less ribbed, but the presence of vascular elements is evident.

With the aid of a microscope and a section of the epidermis, it is clear that the epidermis envelops the tissues of the leaf as a protective layer broken only by stomata and hydathodes. Eames and MacDaniels (1947) suggested that in addition "the epidermis supports the palisade tissue and probably conducts solutes be-

tween the mesophyll cells and the veins." They reported that the apple leaf contains on the lower epidermis approximately 400 stomata per square millimeter which is also an average figure for all mesophytic plants. The stomata are found uniformly over the lower epidermis of the vein islets and usually are lacking on the veins. They are particularly important in photosynthesis studies and have been the subject of much investigation due to their control of gaseous exchange.

Internal. MacDaniels and Cowart (1944) observed that "For the most part apple leaves are remarkably similar in their internal structure, the whole organization being well adapted to the photosynthetic function." Eames and MacDaniels (1947) suggested the reasons:

> 1) the exposure of a large number of chloroplasts to sunlight,
> 2) the exposure of a large cell-membrane surface to the intercellular spaces where interchange of gases takes place, and
> 3) such an arrangement of cells in relation to each other and to the vascular bundles that the products of photosynthesis can be rapidly removed and the cells supplied with water and mineral nutrients.

Between the layers of the epidermis lies the mesophyll, which is itself clearly identified in two parts, the spongy mesophyll and the palisade. Although the distinction is lessened by intermeshing of a few cells from each type of tissue, the number of palisade layers of cells is readily determined. In a leaf cross-section the palisade appears to be more or less a dense tissue. However, a tangential section reveals continuous air spaces among the cells and also continuous connections with the larger air

spaces of the spongy mesophyll. These are the spaces with which Pickett and Kenworthy (1933) were concerned in their correlation of photosynthesis with leaf structure as discussed later.

MacDaniels and Cowert (1944) reported that where there are several layers of palisade cells, they are connected end to end as in filaments. Many of them bend sharply toward the bundle sheaths where the air spaces are reduced and the palisade cells become more compact. They connect directly with the parenchymatous bundle sheath. This connection favors direct conduction of water and nutrients from the vascular system to the mesophyll and the reverse direction for the products of photosynthesis.

Other palisade filaments may connect with the cells of the spongy mesophyll which also form filaments with other spongy cells. A whole network of irregularly oriented filaments thus weave through the air spaces below the vertically-oriented palisade. Those lower spongy filaments attach themselves to the bundle sheaths above or to the smaller bundles below and allow for the translocation process. The Cornell workers reported that the minute bundle ends are intimately connected with all parts of the mesophyll. For the maximum distance recorded between bundle-ends was 88 microns.

MacDaniels and Cowert (1944) suggested that the probable function of vascular bundle sheaths is the prevention of air from entering the water column and disrupting the transpiration pull.

Differentiation. The time required for expansion of apple leaves varies largely with temperature. Usually the first leaves to mature are the basal spur leaves. MacDaniels and Cowert (1944)

reported leaves grown to maturity in five days in 1930. The average length of time required from 2 to 15 days depending upon the location of the leaf on the plant and also the temperature.

In the young leaf prior to the formation of air spaces, the epidermal cells and adjacent subepidermal cells are arranged in regular rows with their long axis perpendicular to the leaf surface. The number of layers of regularly arranged cells on the upper side of the leaf is increased by differentiation of adjacent cells of the central layers of the leaf. The number of layers of palisade tissue varies from one to three depending on size, position of the leaf, and other ecological factors. Cell division ceases while the leaf is yet small, 14-15 mm wide. From then the mesophyll increases in size by an increase in the size of the cells and their spacing. Subsequent growth occurs chiefly in length and breadth rather than in thickness. The palisade layer divisions take place following the final division in the upper epidermis.

In the formation of spongy mesophyll the cells divide at an equal rate with the cells in other parts of the leaf early in the growth process. As division ceases and the leaf expands, the cells become separated and form the characteristic air spaces of the mature spongy mesophyll.

Environmental Influences. The chief factors concerned in leaf structure influences include light, position of the leaf, soil moisture, and inheritance. Cowart (1935) reported that shoot leaves tend to be thicker and larger as compared with leaves from fruiting spurs. Heinicke and Childers (1937) found marked dif-

ference in the shape and thickness of leaves formed at different times. Younger leaves near the terminal of the shoot, they reported as being larger and wider than older leaves. This correlated with their 1933 report that assimilation progressed more rapidly in leaves toward the apex of the shoot. They assumed that water supply was the most important consideration. Pickett (1939) selected leaves for sectioning from middle portions of new shoot growth.

Cowart (1935) reported a decrease in leaf thickness from the base toward the median part of the shoot, and then an increase toward the apex. The palisade cells became more compact, elongate, well defined, and occupied more of the mesophyll as the leaf approached the terminal end of the shoot.

Hanson in 1917 reported that differences in total thickness between leaves on the south edge and center leaves were greater than mesophytic and xerophytic forms of the same species. The southfacing leaves showed more palisade, more compact structure, and thicker epidermis than center leaves.

Correlation to photosynthesis. Research at the Kansas Agricultural Experiment Station over the past seventeen years has included research into the structure and photosynthesis of apple foliage. A chronological listing of the primary conclusions follows.

Kansas Investigations. Pickett (1933) in a preliminary report stated that measurements of differences between the extent of intercellular spaces in the mesophyll of some apple varieties were highly significant. Liveland and Delicious varieties displayed the greatest differences. Compactness of mesophyll was

studied. During the hot dry days of June, 1933, he reported that very few stomata were open after 9 a.m., yet the dry weight increased after that hour. He assumed that carbon dioxide entered the leaf by cuticular absorption.

In 1934, Pickett compared the photosynthetic rates and the extent of intercellular spaces of Liveland and Delicious varieties in the greenhouse and in the orchard. He found that orchard-grown Liveland leaves had more extensive intercellular spaces and more photosynthetic activity than did the Delicious variety. He proposed that the extent of exposed wall surface bordering intercellular spaces possibly was an internal factor helping to regulate the photosynthetic rate.

In 1937, he reported that a greater extent of internally-exposed surface in the Healthy than in the York variety furnished a more extensive moist area on which carbon dioxide was absorbed. Healthy trees made less gain than York trees in the total dry matter produced but showed a greater gain per unit of leaf area. A given amount of chlorophyll, therefore, was capable of a greater production of photosynthate in the Healthy variety.

Pickett and Kenworthy (1939) determined the differences in leaf structure by an actual measurement of the internally-exposed leaf area, using the formula of Turrell (1936), and the externally-exposed leaf area. The relation of the internal to the external area was designated the R value. They concluded that the extent of the internally-exposed surface of apple leaves is more important than the chlorophyll content as a factor partially governing photosynthetic activity.

In 1941 Pickett and Birkeland found that the R ratio was reduced by repeated applications of lime sulfur and lead arsenate. Kenworthy (1939) had found 85% of the internally-exposed surface within the palisade tissue. The assumption, therefore, was made that spray residues may result in altered palisade tissue. This may account for reduced internally-exposed area in sprayed foliage. They suggested further that measurement of the R value could be more simply accomplished by recording direct microscopic measurements of the depth of the palisade tissue.

The same authors in 1942 reported leaf anatomy studies from samples collected three days after the 2nd, 4th, 7th, 8th, 9th, and 10th spray application. They concluded that spray materials shock or check normal cell development with each application. The mild sprays have a less-dwarfing effect than do the stronger material.

In a Kansas Technical Bulletin Pickett and Birkeland (1942) reported a highly significant correlation of 0.88 between the total depth of palisade layers (P), in microns, and the R values. They suggested that application of the regression coefficient 0.1122P 1.33 be applied to the P value as a simplified method of arriving at the R value. They also reported that the lower and central portions of the leaf blade had greater R values than the top and edge portions.

Pickett and Bates (1946) tested seventeen spray materials or combinations of materials for their influence on R values following ten applications. Among other observations, they found that Formate decreased R values on Jonathan foliage. Foliage of

Wealthy, Jonared and Winesap trees sprayed with nitrogenous fertilizers had higher R values than untreated leaves.

In 1947 Bates and Hewett reported that a given treatment may increase the R value and at the same time decrease photosynthesis by its injurious effect without greatly affecting the healthy appearance.

Kwong (1949) commenced a study of organic spray materials on peach leaves and found that Fermate and DDT reduced the depth of palisade cells.

Other Investigations. Turrell (1936) found that the internal exposed leaf surfaces often vary widely from species to species and sometimes between leaves on the same plant. As illustrative of the effects of sunlight and water, he pointed out that the R value was low with 6.8 to 9.9 for shade leaves, intermediate with 11.6 to 19.2 for mesophytes, and high with 17.2 to 31.2 for xeromorphic sun leaves. He suggested that the photosynthetic rate is associated with the moist extensive internal leaf surface. He also explained the correlation of xeromorphic structure and high photosynthetic rate on the basis of high R values in xeromorphic leaves.

Singh and Kal (1935) stated that the morphological classification of leaves is not an index to their photosynthesis. The leaves are more or less one physiological type.

Waugh (1939) reported that under uniform external conditions the rate of assimilation of apple leaves is irregular. He considered internal factors a primary influence. Heinicke, (1933) has found that two leaves growing on the same shoot often vary

considerably from leaf to leaf but the relationship holds from day to day.

Statistical Planning

Potter (1930) discussed the planning of experiments with apple trees. His list of the sources of variability affecting yield and growth in orchard experiments include:

1. Differences in the trees themselves, arising either as heritable differences due to the stock, or from physiological differences due to environmental conditions during the growth of the tree.
2. Differences in the soil, including drainage and moisture supply.
3. Variations in the culture such as pruning, spraying or soil management.
4. Differences in weather conditions.

He urged that the critical worker investigate more simple problems using a more complicated setup rather than the tendency to apply too many treatments with too few applications.

MATERIALS AND METHODS

Plant Materials

All tests reported here were conducted on 30 Winesap apple trees planted March 15, 1946, at the south end of the irrigated college garden for use in transpiration experiments of that year. The investigations for this paper were conducted during the growing season, 1948, and all dates refer to 1948, unless otherwise indicated. The trees were in two east to west rows spaced four feet between rows and three and a half feet apart within the row. A slight gradient from west to east provided ample drainage without

soil erosion. The area was fertile and clean cultivated. Kwong (1949) worked with an adjacent planting of peach trees six feet north of the apple rows.

The year prior to these experiments no spraying nor testing had been attempted on the trees. All of the trees were healthy and pruned to 6 feet on May 8. A serious aphid infestation on June 1, was removed with nicotine sulfate. Canker worm damage to foliage was severe, but prior to the time of experimentation healthy new foliage had replaced it. Due to the variable humidity and temperature, tip-burn was evident from time to time. A mild infestation of apple scab and cedar-apple rust occurred late in July but was not serious enough to affect measurements.

Spray Materials

The concentration of each spray material was prepared as recommended by the manufacturer or as suggested by horticultural literature and general commercial practice. Both the photosynthetic and anatomical portions of this problem received the same concentration of materials. The following materials were used. 1) Chlordane emulsifiable concentrate (45-47 percent), eight fluid ounces in 12½ gallons of water (two pounds in 100 gallons of water) as recommended by the manufacturer, Julius Hyman Company, Denver. 2) A triethylanolamine salt of 2,4-D (2,4-dichlorophenoxyacetic acid) solution prepared at the rate of 10 parts per million as recommended by Harley (1947). 3) Fermate, 1½ pounds in 100 gallons of water, recommended by the manufacturer,

the Dupont Co. 4) Marathon (Thiophos 3422, a 15 percent wettable powder), 1 pound in 100 gallons of water, recommended by the manufacturer, the American Cyanamid Company.

The spray materials were prepared in the laboratory using tap water. A stock solution of 1000 parts per million was prepared for the 2,4-D and brought to the 10 parts per million concentration by dilution when needed. Dermate, Chlordane, and Marathion were prepared fresh at each spraying. Solutions were prepared in Mason quart jars for ease in handling.

Spray Application Equipment

Spray application equipment consisted of an army surplus compressed air tank sprayer modified for use in this problem. The valve-handle was detached from the rod-applicator and soldered to the metal cap from a Mason quart jar. A small home perfume-type atomizer was then soldered to the jar cap and to the valve handle. The use of the soldered jar cap apparatus made it possible to utilize the tank entirely as a compressed air tank. It eliminated washing the large tank every time a different spray material was to be applied. The spray solutions, prepared in separate quart jars, were merely screwed into the soldered jar cap when ready for use. The compressed air was then introduced into the atomizer system and a finely-atomized spray evolved under pressure. Contamination of subsequent spray materials using this same apparatus was lessened by a scrubbing rinse in tap water.

The short rather stiff hose connecting the large air tank

with the valve-handle was replaced with a twelve-foot rubber hose of smaller diameter and lighter weight. The increased length and lighter hose made it possible to walk a radius of 12 feet from the air tank after pumping up pressure. This was sufficient to walk completely around the tree being sprayed and eliminated moving the air tank every few steps.

Contamination caused by drift of air-borne spray materials was lessened by an enclosure of canvas around each tree as it was being sprayed. The enclosure was constructed from war-surplus army "pup" tents.

Application was made at the rate of about 500 to 700 cubic centimeters a tree so that the underside as well as the top of the leaf was covered and dripping.

Statistical Planning

With an elementary understanding of statistical procedure, it seemed advisable to construct methods of design and sampling that would lend themselves and their subsequent data to a critical statistical analysis. Accordingly, with the advise of the experiment station statistician a randomized block design was selected. Together, the investigator and the statistician assigned the order of treatments within each of the six blocks. This randomization was achieved from a book of random numbers and assigned to trees in order from west to east.

Each block consisted of five consecutive trees, comprising one-third of a row. See Fig. 1. Each tree within the block received a coverage of Fermate, Chlordane, 2,4-D, Parathion, or

NORTH ROW	1	5	2	3	4	1	3	4	5	2	3	1	4	5	2	
				Block IV				Block V				Block VI				
SOUTH ROW	4	5	3	1	2	1	4	5	3	2	3	1	4	2	5	
				Block I				Block II				Block III				

Tree 1 - Check, unsprayed Tree 3 - Parathion treatment

Tree 2 - 2,4-D treatment Tree 4 - Chlordane treatment

Tree 5 - Fermate treatment

Figure 1. Randomization of treatments, one per tree, in two east-west rows of Winesap apple trees, college horticultural garden, 1948.

check. The check was simply an unsprayed tree in each block for the purpose of treatment comparison.

Sampling

Photosynthesis. Each block received its spray treatments approximately thirty-six hours prior to the expected time of first sampling. Table 1 indicates the dates of spraying and sampling. All sampling was accomplished as scheduled with the exception of blocks four and five. A rainfall of 0.32 inch two hours following the July 21 spraying was considered a possible nullifying effect. These same blocks were sprayed on the 23rd with subsequent sampling on the 25th. Block one was sampled for two consecutive days, July 1 and 2. Block two was sampled for two consecutive days, July 6 and 7. Blocks three and six were sampled at the same time for two consecutive days, July 15 and 16. Blocks four and five were sampled at the same time for three consecutive days, July 25, 26, and 27.

Sampling with the Ganong leaf punch commenced between 4:30 and 5:30 a.m. so that all punches were completed just as the sun was appearing above the horizon or by 8:15 in two of the blocks. Evening sampling commenced between 3:30 and 4:30 p.m. and finished by 6 p.m. The evening punch on any one leaf was made in the same relative position on the opposite side of the mid-vein from the morning punch. The first-day set of morning and evening punches was made as near to the apex as possible. For the average Winesap leaf this allowed sufficient area for two or three days of additional sampling.

Table 1. Summary of weather information during periods of dry-weight measurement, July, 1940.

Block	Sprayed	Punched			Temperature (°F)		Character of daylight hours
		Date	a.m.	p.m.	Maximum	Range 8 a.m.-4 p.m.	
I	June 29, 5 p.m.				86	70 - 85	Cloudy
		July 1	4:30-630	4:30-6:20	91	70 - 88	Full sun
		July 2	4:40-620	4:35-5:45	88	70 - 85	Full sun
II	July 4, 6 p.m.				89	75 - 89	Partly cloudy
		July 6	4:45-615	4:00-5:15	93	85 - 90	Full sun
		July 7	[illegible]	3:35-4:30	93	80 - 85	Full sun
III, VI	July 13, 6 p.m.				91	85 - 91	Full sun
		July 15	4:45-615	3:30-4:45	88	85 - 88	Full sun
		July 16	4:45-615	3:30-4:45	84	70 - 83	Cloudy
IV, V	July 23, 5 p.m.				79	65 - 79	Full sun
		July 25	5:30-815	3:10-510	91	80 - 91	Partly cloudy
		July 26	5:30-815	3:10-510	89	80 - 87	Cloudy
		July 27	5:30-815	3:10-510	91	75 - 91	Cloudy

Additional notations:

Rain most of the week, July 18 - 20.
.32 inch of rainfall two hours after spraying blocks IV and V on July 21. No punches were taken July 23. To offset possible nullifying effect of the July 21 rain, the trees were sprayed again on the 23rd and punched on the 25th.
Apple scab commenced a serious foliage condition, July 24.

In order to eliminate a source of sampling error, each leaf received both a morning and an evening punch. As an aid to the actual punching procedure, 100 cardboard tags 1" x 1½" were numbered from one through one-hundred and attached to the petioles of 100 representative leaves on the tree. As many leaves as possible received tags on the south side of the tree in continuous sunlight. Tags were attached methodically so that the numbers were easily read and punches could be taken consecutively without danger of missing a single leaf. Each tree required one-half hour for tagging.

A cylindrical metal holder attached to the punch collected the leaf samples. When the 100 leaves of each tree had been sampled, the holder was unscrewed from the punch and the contents transferred into glass vials approximately 2 3/8 x 7/8 inch. The vials had been previously weighed and corks numbered for identification. One set of punches included 100 leaves from each of five treatments in a block.

Oven-drying of the punched material proceeded at approximately 100° C. for a period not less than 24 hours. Following transfer to a desiccator for several hours each vial and contents was weighed on a chainomatic balance and the total weight for 100 leaves recorded. This dry weight represented the sampling of one tree within one block for either the morning or afternoon set of punches. Subtraction of the morning dry weight from the evening dry weight gave weight in milligrams of dry matter accumulated over the loss of photosynthate by respiration and translocation. This weight difference represented the sample datum for one

treatment within one block for one daylight period.

When the weather and leaf areas permitted, samples continued to be collected on subsequent days from the same set of previously-punched leaves. This provided an opportunity to compare weight differences between the afternoon punch and the next morning punch in an attempt to arrive at the night respiration rate.

Internal Structure. The trees and spray materials are identical as described above. The method of application of the spray materials differs from the previous discussion in that tagged leaves alone were saturated rather than spraying the entire tree. On July 13, seven to ten unfolding terminal leaves per tree were saturated by spraying with their specific treatments. At weekly intervals, on the 21st, and the 28th, additional sprays were applied in late afternoon. On August 11, 1948 the tagged leaves were sampled. Leaf samples consisted of a section approximately one by three centimeters taken near the midrib and midway between the basal and apical regions. The tagged leaves, being four weeks old, were by this time located near the middle position on the new shoots.

Killing the leaf samples was hastened by dropping the freshly-cut sample immediately into vials containing killing solution. The procedure for fixing and embedding followed the tertiary-butyl alcohol method of Johansen (1940) except for the paraffin-oil step which was omitted. Satisfactory sections resulted from the use of plaster-of-Paris rectangular molds without concern for the orientation of the leaf surface in the paraffin. Since

permanent slides were not essential, a cementing substance such as balsam was unnecessary. Instead, just prior to use with the microscope, the slide with paraffin ribbon was dipped into xylene and a cover glass applied. Xylene dissolved the paraffin from the tissue from five-to-ten minutes, long enough to make and record observations. For purposes of observation the tissues were clear enough without stain. However, for photographing a stained permanent mount was made.

Leaf cross sections were made at a thickness of 10 microns. A razorblade holder was designed and manufactured at the college metalworking shops for easy interchange of razorblades in the Spencer microtome.

Measurement of the depth of the palisade layers was made with an ocular micrometer which had been previously calibrated with a stage micrometer. Each unit division on the ocular represented 1.695 microns. Along a centimeter-long section of leaf twenty sample readings were made and recorded for each slide. The recorded observation was the linear distance between the upper end of the topmost layer of palisade and the lower end of the lowest layer. For projection of slides upon a photographic plate the apparatus included a single-barreled microscope. Projection equipment appears in Plate III.

Each slide contained a paraffin ribbon of several cross sections from one leaf, but only one section on a slide was sampled. The mean of twenty measurements represented the sampling measurement for each slide. Four leaves were sampled from each tree.

PRESENTATION OF DATA

Two distinct types of measurement have provided the experimental data which follow. Leaf punch increments of dry weight provided for an analysis of treatment effects upon photosynthesis. Microscopic readings provided for an analysis of treatment effects upon internal leaf structure. Observation of both sets of data showed much variation among samples and little evidence of consistent treatment effects. Analyses of variance following Patterson (1939) and Snedecor (1938) have made possible observations that might otherwise have been overlooked.

Photosynthesis

Day Gain. Analysis of the data in Table 3 indicated the relative effectiveness of the four treatments in influencing photosynthetic rates. Table 3 as summarized from Table 2 includes dry weight differences for two consecutive days for Blocks I, II, III, and VI, and for three consecutive days for Blocks IV and V. The measurements for this third day were collected with the intention of using as many data from any one block as leaf surface would allow. However, since all other blocks were punched for two consecutive days only, an analysis was made in which the third-day data was disregarded. This eliminated a bias of the interaction, treatment x block. The analysis of variance appears as Table 4.

The analysis of variance revealed that the variance due to days within blocks was highly significant. The calculated F

Table 2. Daily variation in total dry matter per square meter of Winesap leaf area, 1948.

Block I	Grams per square meter of leaf area				
	Treatment				
	Chlorate	Nitrate	Irrigation	Acid	Check
July 1					
a.m.	87.66	85.52	85.38	91.11	89.74
p.m.	90.58	90.30	88.11	92.62	94.19
Day gain	2.92	4.78	2.73	1.51	4.45
Night loss	- 4.44	-7.01	-8.59	-8.77	-11.38
July 2					
a.m.	86.14	83.29	79.52	83.85	81.31
p.m.	92.65	88.48	84.23	91.94	86.96
Day gain	6.51	5.19	4.71	8.09	5.65

Table 2. (Cont.)

Block II	Grams per square meter of leaf area				
	Treatment				
	Chlorate	Formate	Fumigation	2,4-D	Check
July 6					
a.m.	86.11	82.81	92.41	87.84	85.74
p.m.	92.48	86.53	94.78	94.43	89.71
Day gain	6.37	3.72	2.57	6.59	3.97
Night loss	-7.39	-7.46	-10.63	-9.87	-8.44
July 7					
a.m.	83.09	79.07	84.15	84.56	81.27
p.m.	86.53	83.67	86.80	89.87	82.37
Day gain	3.44	4.60	2.65	5.31	1.10

Table 2. (Cont.)

Block II	Grams per square meter of leaf area				
	Treatment				
	Chlordane	Parathion	Parathion	2,4-D	Check
July 15					
a.m.	90.51	81.21	83.44	87.42	88.54
p.m.	95.84	85.30	93.98	91.84	93.45
Day gain	5.33	4.09	4.54	4.42	4.91
Night loss	-7.35	-5.27	-7.58	-7.32	-8.09
July 16					
a.m.	88.49	80.03	86.40	84.52	87.38
p.m.	92.33	85.80	87.47	84.68	88.38
Day gain	3.84	5.77	0.87	0.16	1.00

Table [illegible] (cont.)

Block IV	[illegible] per square meter of leaf area				
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
July 25					
a.m.	[illegible]	[illegible].35	[illegible].31	[illegible]3.80	87.78
p.m.	[illegible].01	[illegible].11	[illegible]	84.[illegible]	91.67
Day gain	1.32	3.[illegible]3	3.19	1.[illegible]	3.[illegible]
Night loss	-3.02	-[illegible].67	-2.73	-5.[illegible]7	-5.[illegible]
July 2[illegible]					
a.m.	[illegible]	7[illegible].51	[illegible]3.77	7[illegible].[illegible]1	85.78
p.m.	[illegible].5[illegible]	[illegible].32	[illegible]5.31	87.40	91.72
Day gain	[illegible].5[illegible]	7.[illegible]1	1.54	8.49	5.94
Night loss	-[illegible].37	-[illegible].23	-[illegible].77	-9.61	-9.21
July 27					
a.m.	[illegible]	[illegible]	78.54	77.79	82.51
p.m.	87.79	[illegible].30	[illegible]3.29	[illegible]3.27	[illegible]
Day gain	[illegible].58	1.30	4.75	5.48	7.35

Table 2. (Cont.)

Block V	Grams per square meter of leaf area				
	Treatment				
	Chlordane	Fermate	Parathion	2,4-D	Check
July 25					
a.m.	78.88	86.49	85.47	87.31	83.59
p.m.	83.68	89.50	88.65	90.06	87.78
Day gain	4.80	3.01	3.18	2.75	4.19
Night loss	-4.63	-7.26	-5.22	-3.73	-6.70
July 26					
a.m.	79.05	82.24	83.43	86.33	81.08
p.m.	84.16	87.21	86.35	89.45	85.01
Day gain	5.11	4.97	2.92	3.12	3.93
Night loss	-8.36	-3.09	-7.68	-5.27	-6.89
July 27					
a.m.	75.80	84.12	78.67	84.18	78.12
p.m.	81.92	87.58	85.33	89.95	84.69
Day gain	6.12	3.46	6.66	5.77	6.57

Table 2. (Concl.)

Block VI	Grams per square meter of leaf area				
	Treatment				
	Chlordane	Formate	Parathion	2,4-D	Check
July 15					
a.m.	92.82	88.87	94.68	88.19	94.22
p.m.	98.86	97.28	97.57	98.32	99.53
Day gain	6.04	8.41	2.89	10.13	5.31
Night loss	-7.80	-7.07	-6.64	-8.33	-9.03
July 16					
a.m.	91.06	90.21	90.93	89.99	90.50
p.m.	93.78	95.09	92.57	92.06	92.55
Day gain	2.72	4.88	1.64	2.07	2.05

Table 3. Daytime increase in total dry matter per square meter of Winesap leaf area, 1948.

Block	Date	Grams per square meter of leaf area Treatment*				
		C	F	P	D	CK
I	July 1	2.92	4.78	2.73	1.51	4.45
	July 2	6.51	5.19	4.71	8.09	5.65
II	July 6	6.37	3.72	2.57	6.59	3.97
	July 7	3.44	4.60	2.65	5.31	1.10
III	July 15	5.33	4.09	4.54	4.42	4.91
	July 16	3.84	5.77	0.87	0.16	1.00
IV	July 25	1.32	3.83	3.19	1.08	3.88
	July 26	2.59	7.81	1.54	8.49	5.94
	July 27	5.58	1.30	4.75	5.48	7.35
V	July 25	4.80	3.01	3.18	2.75	4.19
	July 26	5.11	4.97	2.92	3.12	3.93
	July 27	6.12	3.46	6.66	5.77	6.57
VI	July 15	6.04	8.41	2.89	10.13	5.31
	July 16	2.72	4.88	1.64	2.07	2.05

* C,Chlordane; F,Formate; P,Parathion; D,2,4-D; CK,Check

Table 4. Analysis of variance of the day gain in total dry matter of Winesap leaf area, 1948, omitting July 27 data.

Sources of Variation	D/F	Sums of Squares	Mean square	F	Probability .05	Probability .01
Total	59	254.325				
Between treatments	4	35.071	8.768	3.138	2.78	4.22
Between blocks	5	10.525	2.105	-----		
Treatments x blocks	20	50.213	2.511	-----		
Days within blocks	6	91.471	15.245	5.457	2.51	3.67
Error*	24	67.046	2.794			

* Treatments x days within blocks

value of 5.45 surpassed the required value of 3.67 at the probability level of one percent. Such significant differences between weight samples within blocks between two consecutive days suggests an active weather influence or a response to injury or to spray materials after the first-day punch. Weather data are presented in Table 1.

Differences between treatment effects were significant at the three percent level of probability. The differences therefore were greater than what might be expected as a result of random sampling alone.

Block differences and the interaction, block x treatment were both nonsignificant. This was to be expected, for the block merely represented an arbitrary grouping of trees and was not assigned on the basis of known soil differences.

A test for the least significant difference (LSD) between treatment means indicated 1.408 gm. as the LSD for the means of all 12 samples. Applying the LSD, Parathion dry weights were lower than Formate, Chlordane, and 2,4-D dry weights. Formate with the highest treatment mean missed being significantly greater than the check, the next highest mean, by the slight margin of 0.186 gm.

Using only the treatment means of samples taken the first day in each block and a LSD of 1.991 gm., there were no significant differences among treatments. Although the observed difference between the Formate and the Parathion means appeared to be large enough for significance, the LSD test failed to establish significance by 0.438 gm.

Using only the treatment means from samples taken the second day in each block and a LSD of 1.991, 1) the Formate treatment established superiority over the check, and 2) the Formate and the 2,4-D treatments were both significantly greater than the Parathion treatment.

The observed order of treatment means remained consistent throughout the comparisons of the means of total, first-day, and second-day punches, except for a slight reversal of the 2,4-D, check, and Chlordane means within the first-day set. Such a slight reversal of order was within reason, for at no time were there significant differences among the three treatments.

LSD comparisons used the treatment means in Table 5.

Since the first analysis of variance established the interaction, treatment x block as being even smaller in magnitude than the sampling error, a second analysis of variance was prepared. All seventy dry weight samples including July 27 data were used. The analysis appears as Table 6. Again, the variance of the factor, consecutive-days-in-blocks surpassed the required 3.12 value at the one percent level of probability with a computed 4.19 value. All other factors proved nonsignificant.

Contrary to what was expected on the basis of the first analysis treatment differences for the second analysis were no greater than what might be expected as a result of random sampling alone. For five percent probability an F value of 2.67 was required. The computed value was 1.60. The discrepancy between the significant treatments of the first analysis and the nonsignificant treatments of the second analysis presumably rests

Table 5. Treatment means of day gain in dry weight for first-day, second-day, and pooled data.

Treatment	Grams per square meter of leaf area		
	Mean of total	First-day mean	Second-day mean
Fermate	5.088	4.640	5.537
2,4-D	4.477	4.413	4.540
Chlordane	4.249	4.463	4.035
Check	3.866	4.455	3.353
Parathion	2.786	3.183	2.390

Table 6. Analysis of variance of the day gain in total dry matter of Winesap leaf area, 1948, including July 27 data.

Sources of Variation	D/F	Sums of squares	Mean square	F	Probability .05	Probability .01
Total	69	295.249				
Between treatments	4	21.097	5.274	1.60	2.67	3.97
Between blocks	5	9.681	1.936	----		
Treatments x blocks	20	56.168	2.808	----		
Days within blocks	8	106.579	13.322	4.191	2.25	3.12
Error*	32	101.724	3.179			

* Treatments x days within blocks

in the data for July 27. There appears to be no reason why these extra data for one day should affect the significance unless there were a sampling error or an unknown factor at work. There are insufficient data available to determine the source of the conflict. Observation of July 27 treatment means, however, indicates that the Fermate samples did not follow the relative

ranking of the total treatment means as listed in Table 7.

Table 7. Treatment means of day gains in dry weight for July 27 and means for all samples.

Treatment	Total means		July 27 Samples	
	Excluding July 27	Including July 27	Block IV	Block V
Formate	5.088	4.701	1.30	3.46
2,4-D	4.476	4.641	5.48	5.77
Chlordane	4.249	4.477	5.58	6.12
Check	3.904	4.308	7.35	6.57
Parathion	2.786	3.202	4.75	6.66

The Formate weights in both Blocks IV and V are the smallest of all the treatment means, whereas the ranking should be up near first place as compared with several sets of treatment means, Table 7 and Table 8. Comparisons of other treatment means show deviations, also.

Night Loss. The nightly difference in dry weights between the morning of the second day's punches and the punches of the previous evening are listed in Table 9 as summarized from Table 2. These data consistently represented losses in dry matter during the absence of photosynthesis and can therefore be assigned to translocation and respiration usage. Since photosynthesis and respiratory processes are complexly intermeshed, these data are presented for their contribution to a more complete understanding of the total data.

Table 8. Treatment means of day gain in dry weight expressed in grams per square meter of leaf area and grouped for ready comparisons.

Average of All Samples	excluding July 27	including July 27
Fermate	5.088	4.701
2,4-D	4.477	4.641
Chlordane	4.249	4.477
Check	3.866	4.308
Parathion	2.786	3.202

Block IV, V, - Third Day Average of two samples	
Check	6.96
Chlordane	5.85
Parathion	5.71
2,4-D	5.62
Fermate	2.38

First Day Samples All Blocks	Average of six samples
Fermate	4.640
Chlordane	4.463
Check	4.455
2,4-D	4.413
Parathion	3.183

Second Day Samples All Blocks	Average of six samples
Fermate	5.237
2,4-D	4.540
Chlordane	4.035
Check	3.353
Parathion	2.360

July 15 Blocks III, VI	Average of two samples
2,4-D	7.27
Fermate	6.25
Chlordane	5.68
Check	5.11
Parathion	3.72

July 16 Blocks III, VI	Average of two samples
Fermate	5.32
Chlordane	3.28
Check	1.52
Parathion	1.25
2,4-D	1.11

July 25 Blocks IV, V	Average of two samples
Check	4.04
Fermate	3.42
Parathion	3.18
Chlordane	3.06
2,4-D	1.91

July 26 Blocks IV, V	Average of two samples
Fermate	6.39
2,4-D	5.81
Check	4.93
Chlordane	3.85
Parathion	2.23

Table 9. Night loss in total dry matter per square meter Winesap leaf area, 1948.

Block	Date	Grams per square meter of leaf area — Treatment*				
		C	F	P	D	CK
I	July 1	4.44	7.01	8.59	8.77	12.88
II	July 6	9.39	7.46	10.63	9.87	8.44
III	July 15	7.35	5.27	7.58	7.32	6.07
IV	July 25	3.02	8.67	2.73	5.97	5.89
V	July 25	4.63	7.26	5.22	3.73	6.70
VI	July 15	7.80	7.07	6.64	8.33	9.03
	Average	6.11	7.12	6.89	7.33	8.17

* C,Chlordane; F,Fermate; P,Parathion; D,2,4-D; CK,Check

An analysis of variance, Table 10 reveals that variations between treatments were nonsignificant with a computed F value less than 1.0. Variations between blocks were significant at the three percent level of probability. Since the block was merely an arbitrary grouping of trees and not a grouping based upon soil differences, this significance was due to tree variability or to weather factors. In the foregoing analyses of daytime data the weather variation was considerable. It is assumed that the weather factor again operated on this evening data. Table 11 presents temperature data relative to this period of sampling.

A LSD of 2.480 gm. was applied to the means of the block totals. Block I and Block III punched on different days were both significantly greater than both Blocks IV and V punched the

Table 10. Analysis of variance of the night loss in total dry matter of Winesap leaf area, 1948.

Sources of Variation	D/F	Sums of squares	Mean square	F	Probability .05	Probability .01
Total	29	145.60				
Between treatments	4	133.38	3.334	-----		
Between blocks	5	61.49	12.299	3.476	2.71	4.10
Treatments x blocks	20	70.77	3.536			

Table 11. Nighttime temperatures during periods of dry-weight measurement, 1948.

Block	Date	Temperature (°F) Minimum	Temperature (°F) Range 6 p.m.-8 a.m.
I	6 p.m. July 1 to 8 a.m. July 2	65	70 - 65
II	6 p.m. July 6 to 8 a.m. July 7	55	75 - 55
III, VI	6 p.m. July 15 to 8 a.m. July 16	68	75 - 68
IV, V	6 p.m. July 25 to 8 a.m. July 26	68	80 - 68

same day. The mean for Block II was sufficiently different from Block III for significance

Leaf Structure

Table 13 presents the R values for microscopic observations of P values. The conversion from P to R values involved the correlation factor (.1122P + 1.33) derived by Pickett and Birkeland (1942). R values for the missing Check P values, Block III were obtained by using the equation recommended by Patterson (1939),

page 162. One missing value for Chlordane, Block I required an R value averaged from the three other R values within the block.

An analysis of variance Table 12 revealed a highly significant interaction variance, treatment x block. The treatment variance was considerably higher than the block variance but not enough to reveal a consistent treatment effect among all blocks. The interaction variance indicated that the relative ranking of treatment effects changed from block to block. A nonsignificant but high value for the treatment variance substantiates the fact that there were significant differences within any one block but inconsistent ranking from block to block.

A LSD applied to the treatment averages within each block revealed the significant differences noted in Table 14. Two of the six blocks showed nonsignificance for any treatment. The relative ranking of treatment means varied considerably. Fermate in two blocks was greater than the check, and in one block was less than the check. In two blocks Chlordane, Fermate and 2,4-D were all greater than the check and Parathion. In one instance the check was greater than Parathion. Among significantly different comparisons the Parathion treatment was the most consistently low. This agrees with the order of rank as displayed by the means of the totals for all treatments.

Treatment	Mean R Value
Chlordane	13.10
2,4-D	12.77
Fermate	12.61
Check	12.17
Parathion	11.55

Table 12. Analysis of variance of R values of Winesap apple foliage, 1948.

Sources of variation	D/F	Sums of squares	Mean square	F	Probability .05	Probability .01
Total	114	312.36				
Between treatments	4	36.35	9.087	1.36	2.90	
Between blocks	5	30.36	6.072	----	----	
Treatments x blocks	19	126.67	6.667	4.83	1.70	2.11
Error	86	118.98	1.38			

Table 13. Means of 20 readings of the depth of palisade and corresponding R values for treatments on Winesap apple foliage, 1948.

Block	Treatment					
	Parathion		2,4-D		Chlordane	
	P value (microns)	R value*	P value (microns)	R value*	P value (microns)	R value*
I	81.36	10.46	89.50	11.37	138.65	16.89
	76.61	9.93	105.01	13.12	104.75	13.08
	85.09	10.88	124.75	15.33	110.51	13.73
	100.68	12.63	117.97	14.57		14.57
Average		10.97		13.59		14.56
II	89.50	11.37	108.48	13.50	100.01	12.55
	95.94	12.09	101.36	12.70	111.53	13.84
	89.84	11.41	120.01	14.80	93.23	11.79
	87.80	11.18	87.80	11.18	118.99	14.68
Average		11.51		13.04		13.21
III	94.92	11.98	90.51	11.49	121.36	14.95
	104.07	13.01	109.50	13.62	114.92	14.22
	104.07	13.01	91.87	11.64	107.42	13.39
	107.80	13.43	87.80	11.18	92.21	11.68
Average		12.85		11.98		13.56
IV	92.21	11.68	88.82	11.30	102.38	12.82
	87.46	11.14	68.14	8.98	88.82	11.30
	76.28	9.89	77.63	10.04	81.02	10.42
	103.06	12.89	86.78	11.07	81.39	10.69
Average		11.40		10.34		11.30
V	97.97	12.32	95.60	12.06	98.99	12.44
	113.57	14.07	105.43	13.16	108.14	13.46
	95.26	12.02	92.21	11.68	88.81	11.29
	107.46	13.39	97.29	12.25	91.53	11.60
Average		12.95		12.28		12.19
VI	72.55	9.47	113.57	14.07	116.96	14.45
	82.04	10.53	112.55	13.96	124.07	15.36
	64.75	8.59	123.40	15.18	94.24	11.90
	75.26	9.77	125.21	18.41	117.29	14.49
Average		9.59		15.40		14.05

Table 13. (Concl.)

Block	Treatment: Fermate: P value (microns)	Fermate: R value*	Check: P value (microns)	Check: R value*
I	115.94	14.34	97.63	12.28
	137.30	16.74	86.11	10.99
	114.58	14.19	90.85	11.43
	106.11	13.24	87.12	11.10
Average		14.62		11.45
II	85.09	10.88	95.60	12.06
	78.31	10.12	97.97	12.32
	83.73	10.72	110.18	13.69
	96.28	12.13	106.79	13.31
Average		10.96		12.84
III	104.07	13.01		12.44
	99.67	12.51		12.44
	95.26	12.01	Missing**	12.44
	108.48	13.50		12.44
Average		12.75		12.44
IV	96.95	12.21	100.34	12.59
	100.68	12.63	100.68	12.63
	82.72	10.61	99.33	12.47
	94.58	11.94	93.23	11.79
Average		11.84		12.37
V	90.85	11.52	117.63	14.53
	93.56	11.83	98.99	12.44
	95.94	12.09	95.60	12.06
	109.16	13.58	86.45	11.03
Average		12.25		12.51
VI	114.24	14.15	92.89	11.75
	85.77	10.95	81.70	10.50
	104.75	13.08	88.14	11.22
	119.67	14.76	96.95	12.21
Average		13.23		11.42

* (0.1122P) + 1.33

** See page 53

Table 14. Treatment means and significant treatment differences of R values within blocks of Winesap apple foliage, 1948.

Block	Treatment	R value	Significant treatment differences (LSD 1.66)
I	Formate	14.62	Formate greater than check, Parathion
	Chlordane	14.56	Chlordane greater than check, Parathion
	2,4-D	13.59	2,4-D greater than check, Parathion
	Check	11.45	
	Parathion	10.97	
II	Chlordane	13.21	Chlordane greater than Parathion, Formate
	2,4-D	13.04	2,4-D greater than Formate
	Check	12.84	Check greater than Formate
	Parathion	11.51	
	Formate	10.96	
III	Chlordane	13.56	
	Parathion	12.85	
	Check	12.44	No differences
	Formate	12.75	
	2,4-D	11.98	
IV	Check	12,47	Check greater than 2,4-D
	Formate	11.84	
	Parathion	11.40	
	Chlordane	11.30	
	2,4-D	10.34	
V	Parathion	12.95	No differences
	Check	12.51	
	2,4-D	12.28	
	Formate	12.25	
	Chlordane	12.19	
VI	2,4-D	15.40	2,4-D greater than Formate, Check, and Parathion
	Chlordane	14.05	Chlordane greater than Check, Parathion
	Formate	13.23	Formate greater than Check, Parathion
	Check	11.42	Check greater than Parathion
	Parathion	9.59	

DISCUSSION OF RESULTS

Photosynthesis

In relation to dry weight measurements of photosynthesis, a treatment dry weight approximating that of the check presumably causes no measurable effect upon vital life processes. An increased dry weight compared with the check denotes a stimulatory effect from the treatment by causing an increase of the actual amount of photosynthate or by slowing down the metabolic or translocatory processes accompanying photosynthesis. A decreased dry weight compared with the check indicates a decrease in the rate of photosynthate manufacture or a stimulating effect upon the accompanying respiration and translocation.

All daytime measurements consistently gained in total dry weight and all nighttime measurements decreased in total dry weight. This fact agreed with the assumption underlying this method of measuring photosynthesis. The daytime and nighttime data were in general agreement. Treatment differences among data from the 36 to 60 hour period following spraying were not significant.

Important differences between dates of sampling appeared in both daytime and evening data. These indicated a possible weather factor such as temperature or sunlight. Blocks IV and V sampled at the same time displayed similar low values on cloudy July 25. Other blocks showed higher values. From this it is assumed that a lesser amount of radiant energy caused less production of photosynthate. The lessened total amount of

photosynthate became a limiting factor, which in turn reduced the respiration rate the following evening.

Following the 60 hour period, differences commenced to appear. Second-day samples and pooled first-day and second-day samples produced differences among the treatments and one difference between a treatment and the check. Aside from active unknown factors there were two possible reasons for the increased activity. First, the spray materials had had more time to react on the foliage and possibly, therefore were approaching the threshhold of a reactive period. Secondly, the injury of the leaf caused by prior punches may have stimulated enzyme activity and the general leaf metabolism. A varying response to different spray materials would have increased the complexity of the problem. If the leaf injury assumption as reviewed by Rabinowitch (1945) is correct, these data reemphasize the inherent error in this method of photosynthesis measurement. A retesting with the CO_2 apparatus as described by Heinicke and Hoffman (1933) or the season gain in dry weight for the whole plant (Pickett, 1942) might indicate the value of the assumption.

An incomplete sampling from all blocks of the consecutive third-day caused reversals of observations made from the two-day data. Treatment differences disappeared. There appeared to be no explanation except as a sampling error. This may have been the reason, for the Fermate samples shifted from the position of highest weight to that of lowest weight samples for that day. A larger leaf area permitting complete sampling of all blocks for a four or five-day period would have provided more evidence for

a valid statement of sampling error. It is possible but not probable that a combination of factors such as time-lapse, leaf injury, temperature, and sunlight may have caused such reversals.

In spite of much variability among the analyses, the complete data for two days provide for valid generalizations of treatment effects. The analyses substantiate conclusions gained from a casual observation of treatment total dry weights for all samples. Statements concerning each treatment follow.

Fermate showed superiority over the check within both the complete and the second-day sets of samples. This superiority is a statistically significant increase in ability to manufacture photosynthate. In all comparisons the Fermate fungicide showed superiority over the parathion insecticide.

2,4-D, Chlordane, and the check showed no great differences in photosynthate manufacture. The 2,4-D and Chlordane treatments, therefore, are assumed to have little immediate detrimental or stimulatory effect upon photosynthesis. Both the Chlordane and the 2,4-D tend to stimulate the process, though not significantly, as compared with the check.

Parathion at no time showed a significant difference from the check although it tended to be lower than the check. Parathion appears to slow down the rate of photosynthesis but probably without an actual detrimental effect to the plant. As compared with the other treatments Parathion definitely produced the least amount of photosynthate. In this respect it is inferior statistically to Fermate, Chlordane, and 2,4-D.

Leaf Structure

The samples of leaf structure represented young unfolding leaves sprayed three times at weekly intervals and then sampled thirty days following the first spray application. The four-week interval allowed for the growth of the leaf to maturity and to a position a foot or more from the end of the rapidly growing twig. The author assumed that one month was sufficient time for abnormal effects of spray materials to become evident on internal structure.

Statistical analysis underscored the observation that no single treatment effect remained consistent, relative to the other treatments, among the blocks. The treatment variance was enough higher, however, to suggest that generalizations are possible within blocks. Two blocks indicated no change in R values. One block showed only one large difference, and three blocks showed at least three significant treatment differences. Blocks I and VI produced similar significant results.

Pickett and Kenworthy (1939) reported that an increase in the R value as compared with the check indicates an increased capacity for manufacturing photosynthate. A decreased R value means a lesser capacity for photosynthesis.

Formate in two blocks increased R values over the check and over Parathion. Block II placed Formate as the significantly lowest treatment. The effect of Formate, therefore, remains uncertain, though probably with a tendency to increase the R values.

The insecticide Chlordane in three blocks produced increased R values significantly greater than the Parathion insecticide. It was superior to the check in two blocks and to Fermate in one block. Chlordane, therefore displays a tendency to actively increase R values.

The 2,4-D displayed variability but appears to increase R values as compared with the check.

Parathion most consistently produced the lowest R values but was significantly different from the check in only one block. This insecticide showed the tendency to decrease the R value and in turn, the photosynthesis of the foliage.

Dry Weight and R Value Compared

The effects of only the Parathion treatment appear consistent enough to justify a definite statement comparing data of dry weights and R values. Since Parathion remained the lowest-rank treatment in both experiments, there is reason to believe that it is less beneficial than the other treatments and perhaps even detrimental to the photosynthetic process. A more extensive testing using the CO_2 absorption method and R value sampling of the same leaves would be desireable.

Further testing of Fermate and Chlordane might reveal real differences that in these limited data appeared merely as tendencies to increase photosynthesis and the R value. 2,4-D appeared to be less different from the check as compared with the other treatments. The value of these experiments appears to be

as a pilot trial charting the course for more extensive experimentation with these newer spray materials.

SUMMARY

1. Application of Chlordane, Fermate, Parathion, and 2,4-D spray materials were compared with untreated trees of the Winesap apple variety for their effects on apparent photosynthesis and the R value of the foliage. The investigations were conducted at Manhattan, Kansas during July, 1948.

2. Using the Canong leaf-punch method for measuring photosynthesis, all daytime measurements consistently gained in total dry weight and all nighttime measurements decreased in total dry weight.

3. The Fermate material increased the capacity of the leaf for photosynthesis. 2,4-D and Chlordane caused only a slightly greater increased rate. Parathion showed a decrease in the rate.

4. Leaf tissue for R value measurement was prepared by paraffin sectioning of leaves which had received four weekly spray applications.

5. Parathion caused a slight decrease in the R value and was the smallest R value observed. The other treatment effects on the R value resembled the check.

EXPLANATION OF PLATE I

Fig. 2. Appearance of leaves after the first, second, third, and fourth punch.

Fig. 3. Tagged leaves following the first set of morning punches.

PLATE I

Fig. 2.

Fig. 3.

EXPLANATION OF PLATE II

Fig. 4. The Canong leaf-punch removing 1 cm sq. area for photosynthesis study.

Fig. 5. Unfolding terminal leaf at time of spray application for anatomical study.

PLATE II

Fig. 4

Fig. 5

EXPLANATION OF PLATE III

Fig. 6. Equipment for taking photomicrographs.

Fig. 7. Internal leaf structure, X 307. Formate treatment.

PLATE III

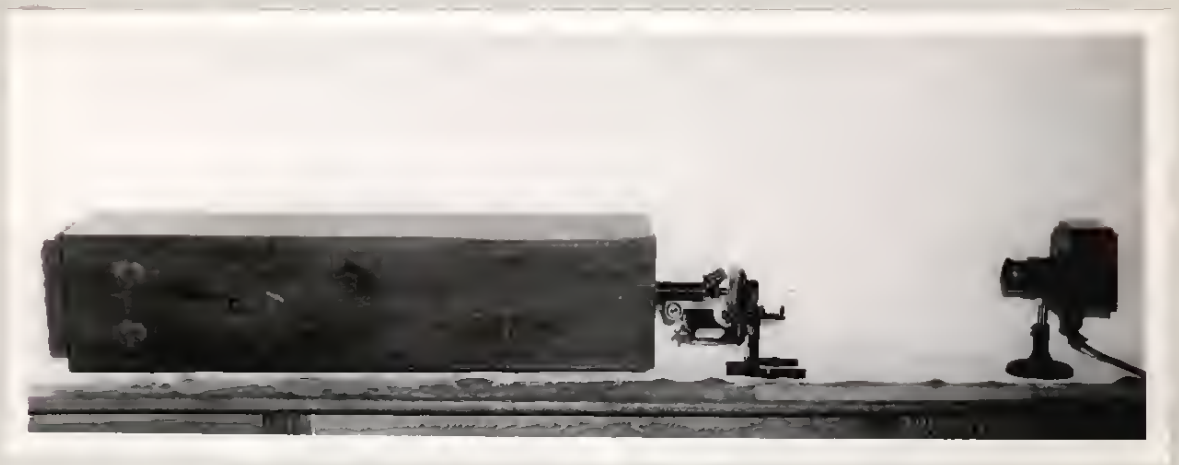

Fig. 6.

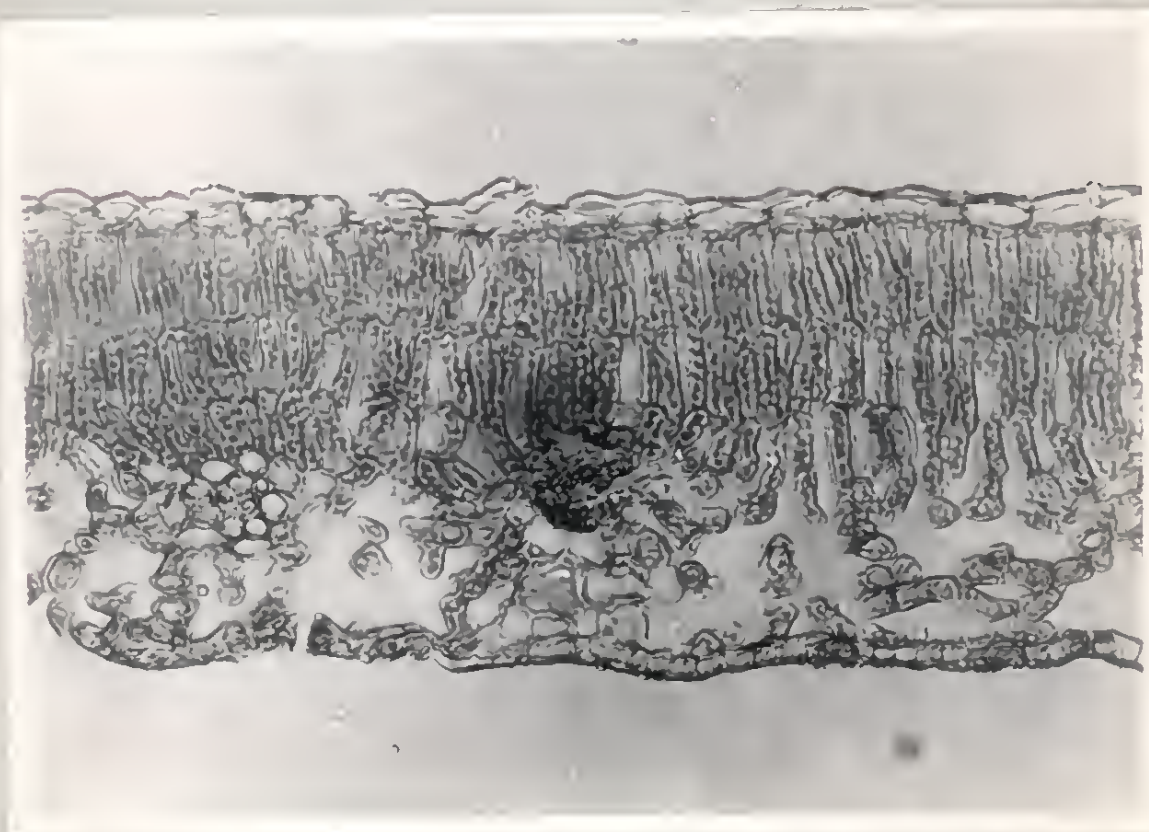

Fig. 7.

EXPLANATION OF PLATE IV

Fig. 8. Internal leaf structure, X 307. Chlordane treatment

Fig. 9. Internal leaf structure, X 307. Parathion treatment.

PLATE IV

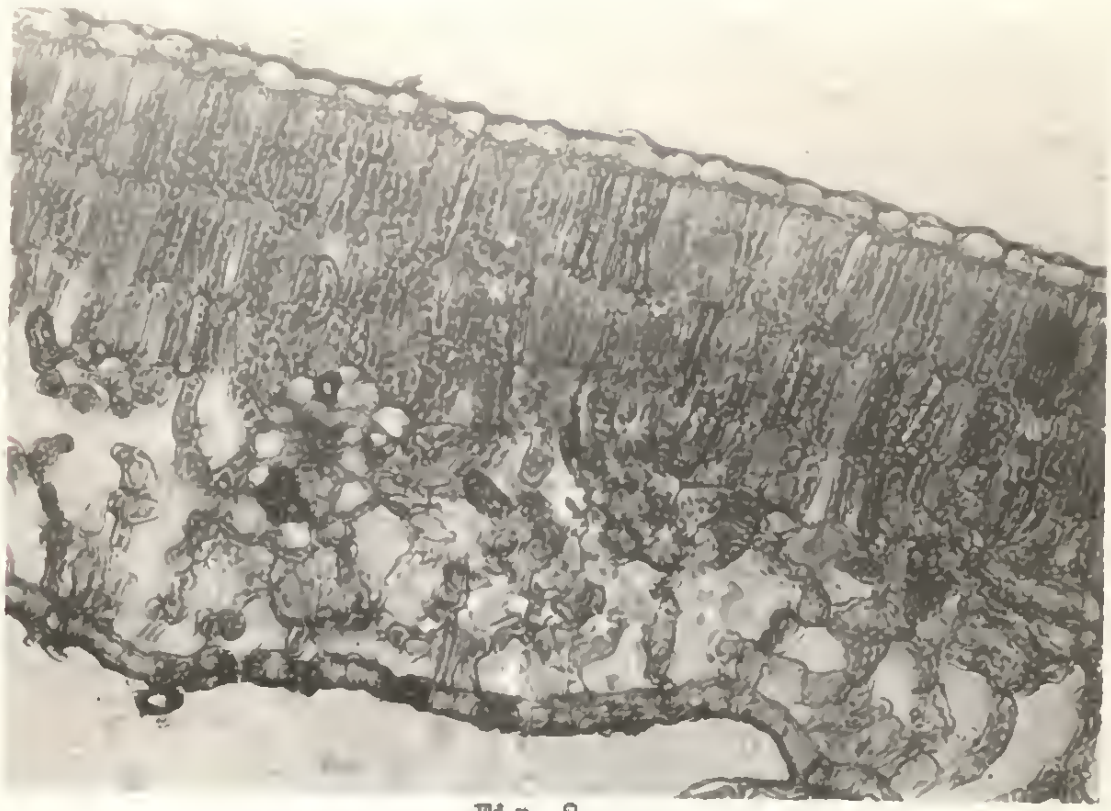

Fig. 8.

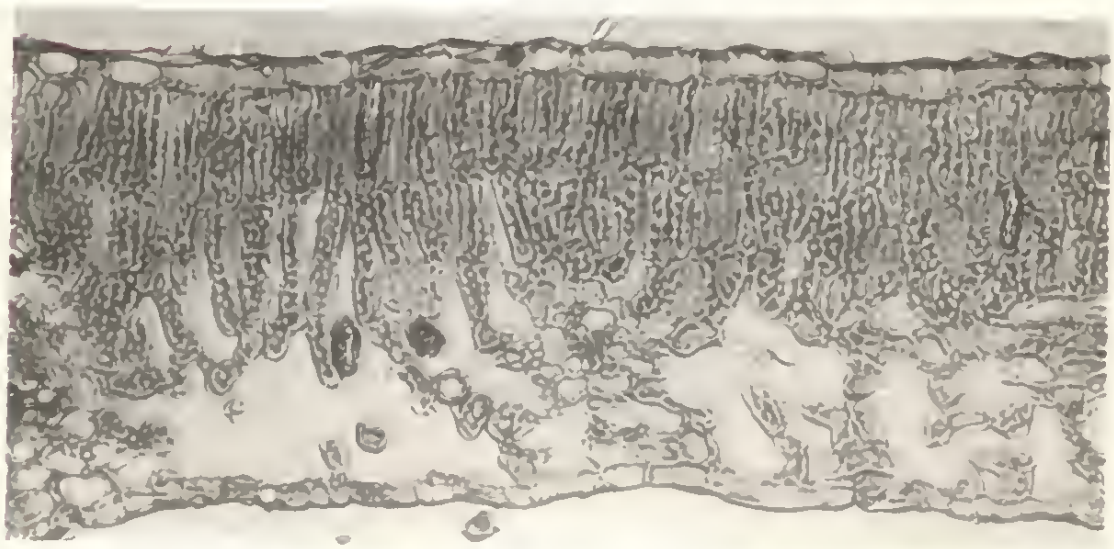

Fig. 9.

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